線虫 Caenorhabditis elegans を用いたストレス応答機構に関する研究(Digest_要約)

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Theanalysis of stress response systems in *Caenorhabditis elegans.*

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Abstract

Backgrounds

The organisms are exposed to various stresses. Oxidative stress is the one of the most common stress. Oxidative stress is induced by excessive accumulation of reactive oxygen species (ROS) such as the superoxide anion radical ($\cdot O^{2-}$), the hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\cdot OH$). ROS cause oxidative damage to DNA, proteins and lipids. To overcome these stresses, stress response systems have been evolved.

There are two systems to overcome oxidative stress, DNA damage response (DDR) and response to ROS. There are many reports about the relationship between stress and cellular dysfunction. However, there is great need for the research at organism level. In this study, I highlighted the two DDR pathways, DNA mismatch repair and ATM related pathways. MMR has been found to act as a correcting system for DNA polymerase errors. However, recent studies revealed that MMR has various functions besides correcting mismatches, DNA damage response and regulation of homologous recombination. In spite of many researches, the details of these pathways are not fully understood, especially contribution at organism levels. ATM is identified as the genes related in ataxia-telangiectasia. ATM participates in many stress response pathways, the response to H_2O_2 and radiation.

Methods

To analyze the contribution of stress response at organism level, we researched various stress response pathway using *Caenorhabditis elegans.*

C. elegans is widely used as a model organism of aging, development and apoptosis.

The adult hermaphrodite contains just 959 somatic cells, but has basic organs like, nerves, intestines and muscles. That number of somatic cells is far fewer than the number of cells in other multicellular model organisms, and these cells do not undergo proliferation. Hence, *C. elegans* is suitable to analyze the role of DDR at the organism level because of its genetically stability during one generation.

Results

In this study, first, I analyzed the affinity of CeMutSα (CeMSH2-CeMSH6 heterodimer). In gel-shift assays, I found that CeMutSα can recognize the two and more nucleotide insertion or deletion. However, unexpectedly CeMutSα did not show an affinity for the single nucleotide insertion or deletion. This fact indicates that CeMutSα has different affinity from deuterostome. In order to analyze these differences, I performed alignment analysis for MutS among deuterostome and protostomes and identified four significant amino acid-changes. Moreover, to test these amino acid-changes I purified an amino acid-variant of CeMutSα and analyzed its affinity. Unfortunately, this amino acid-variant showed an affinity for the two and more nucleotide insertion or delete. However, that recognized the single nucleotide insertion or deletion like MutSα in deuterostome. In addition, interestingly that variant had increased binding affinity for mismatches and different affinity for various DNA lesions. These results suggest that mutations that affect primary structure of MutS may induce abnormal signaling by MMR and disrupt the cell function. In addition, to analyze DDR dunction in MMR, I analyzed the affinity of CeMutSα for various DNA lesions and found that CeMutSα could recognize not only mismatches such as G-T, but also DNA lesions containing 5-formyl uracil, 8-oxoguanine and uracil.

Then, I analyzed the function of mismatch repair at the cellular level in worm gonad arms. The results showed that mismatch repair could induce apoptosis in gonad arms of worms exposed to various DNA damage sources except γ-rays. My findings at the organism level suggested that cell death induced by mismatch repair can lead to the tissue and/or organism death. In addition, my results also suggested that the function of mismatch repair is regulated depending on the tissue or growth stage, and that cell death is induced independently of DNA replication. Hence, I hypothesized that mismatch repair works as a DNA damage sensor and its functions are different depending on its tissue.

In ATM study, in drug sensitivity assays, I found that the growth stage larvae showed the sensitivity to various DNA damaging-agents that induces DNA double strand breaks. These results are corresponding with previous researches with mammalian cultured cells. However, adult worms did not show sensitivity to some of these drugs. These results suggest that the some drugs induce DNA double strand breaks depending on DNA replication. In addition, ATM deficient adult worms showed resistance to H_2O_2. Therefore, I hypothesized that ATM activation by H_2O_2 induces cell death and the sensitivity to oxidation agents of mammalian ATM deficient cells is due to the DNA double strand breaks induced by oxidation.

In addition, to apply these researches to the anti-aging, I examined whether dietary supplementation with herbal mixture could provide protection against oxidative stress, extend lifespan, and delay aging in *C. elegans*. We found that herbal mixture extended lifespan and delayed aging in adult *C. elegans*. The expression of oxidation resistance 1 (OXR1) protein was induced by juglone and this effect was significantly suppressed in herbal mixture-treated. In addition, the amount of oxidized protein was significantly

lower in herbal mixture-treated worms than untreated worms. Furthermore, locomotive activity was increased in *C. elagans* at 3 days of age following the treatment with herbal mixture. On the other hand, the level of cellular ATP was lower at 3 days of age in worms treated with herbal mixture than in untreated worms. Herbal mixture increases lifespan and delays aging in *C. elegans*, well corresponding to its activity to protect against oxidative stress.

Conclusion

I revealed the relationship between DDR and organism death in MMR and ATM. In addition, I succeeded the evaluation of dietary supplementation with herbal mixture in *C. elegans*.